

## PARADOX PHENOMENA IN THE CARDIAC GANGLION OF LIMULUS POLYPHEMUS<sup>1</sup>

IPING CHAO<sup>2</sup>

(From the Marine Biological Laboratory, Woods Hole, and the Department of  
Physiology, University of Chicago)

It was found by Zwaardemaker (1917, 1925) that when a frog's heart is perfused with a Ringer's solution containing 40 mgm. thorium nitrate per liter instead of the 170 mgm. potassium chloride normally present, a temporary arrest of the beat occurs at the moment of replacing the thorium-containing by the potassium-containing Ringer's solution. This temporary inhibition of the activity of the heart on replacing a light radioactive element (potassium and rubidium in one group) by a heavy radioactive element (uranium, thorium, radium, emanation and ionium in another group), or vice versa, was called by Zwaardemaker the radio-physiologic paradox. Shortly afterwards Libbrecht (1920) found that the same temporary inhibition of the frog's heart can be obtained on change from a K-free to a normal Ringer's solution, and he called this striking phenomenon the potassium-paradox. Libbrecht (1921) also described a thermo-paradox in the frog's heart on change from a warm to a cold Ringer's solution. More recently Kisch (1930) reported a Ca-paradox on change from a Ca-free to a normal Ringer's solution and a Sr-paradox on change from a Ca-free to a normal Ringer's solution containing an equivalent amount of SrCl<sub>2</sub> instead of CaCl<sub>2</sub>.

Paradoxes of this kind are found not only in the frog's heart but also in the hearts of other animals and in other tissues. Thus the K-paradox has been described in the rabbit's heart by Busquet (1921, 1922*b*) and in the heart of the spider crab by Wells (1928). Busquet (1922*a*) also observed a similar paradox in the rabbit's heart on change from a K-free Ringer's solution to the same solution containing ammonium chloride. The K-paradox has been demonstrated in the automatic movement of the frog's œsophagus by Bakker (1927) and in the isolated fore-gut of insect by Hobson (1928). It is also found in the ganglion of the *Limulus* heart (Chao, 1933). In this paper a further study of the conditions

<sup>1</sup> A preliminary report of this work appeared in the *Collecting Net*, 1933, 8: 341.

<sup>2</sup> Partly aided by a Collecting Net Scholarship.

for the production of the K-paradox in this ganglion will be presented, together with observations on the Ca-paradox and thermo-paradox.

The experimental procedure has been described in a previous paper (Chao, 1933). Briefly, the ganglion is isolated from the heart musculature posteriorly and the heart is sectioned behind the second segment, leaving the ganglion in connection with the anterior two segments of the heart only. The heart is mounted in sea water for graphic registration of the contractions, which result from impulses originating in the nerve cells of the ganglion; the latter is immersed in a Ringer's solution. The Ringer's solution contains 445 millimols NaCl, 8.9 millimols KCl and 37 millimols  $\text{CaCl}_2$  in a liter. It can be rapidly replaced by another solution without disturbing the ganglion-heart preparation. All experiments (except those on the thermo-paradox) are performed at the room temperature; this fluctuates usually within one degree during an experimental period of two to three hours, and varies between  $22^\circ$  to  $26^\circ$  C. during the whole season.

#### THE POTASSIUM-PARADOX

When a ganglion is immersed in a K-free Ringer's solution, the rate is slightly increased. Ten minutes later the K-free Ringer's solution is replaced by a normal Ringer's solution;<sup>3</sup> the rate and amplitude, instead of returning gradually to normal, are immediately decreased, and in some cases the heart-beat may be even completely arrested. This temporary inhibition is the characteristic phenomenon of the paradox; it lasts for a short period and is followed by a gradual recovery. The extent of the inhibition depends upon several factors, which will be discussed in order.

##### *The Effect of Repeated Immersion in the K-free Ringer's Solution*

In general, the K-paradox is more readily obtained on a second or third repetition than on the first immersion of a fresh ganglion in the K-free Ringer's solution. The following experiment (No. 86), although an extreme case, illustrates this point particularly well. When this ganglion was first immersed in a K-free Ringer's solution for ten minutes, no K-paradox was obtained on return to the normal Ringer's solution. Twenty minutes later the ganglion was again immersed in a K-free Ringer's solution for another ten minutes. Then on change of solutions the rate decreased from 15.5 beats per minute (the rate at the tenth minute in the K-free Ringer's solution) to 5, 6, 6, 8, 8, 8, 8, 9, 9,

<sup>3</sup> The K-paradox can also be demonstrated on changing from a K-free Ringer's solution to sea water.

and 9 in successive minutes in the normal Ringer's solution; after twenty minutes the rate was 9.5. Corresponding to the primary decrease in rate, there was also a slight decrease in amplitude (Fig. 1, *a*). These changes in rate and amplitude were even more pronounced after the third immersion in the K-free Ringer's solution for the same length of

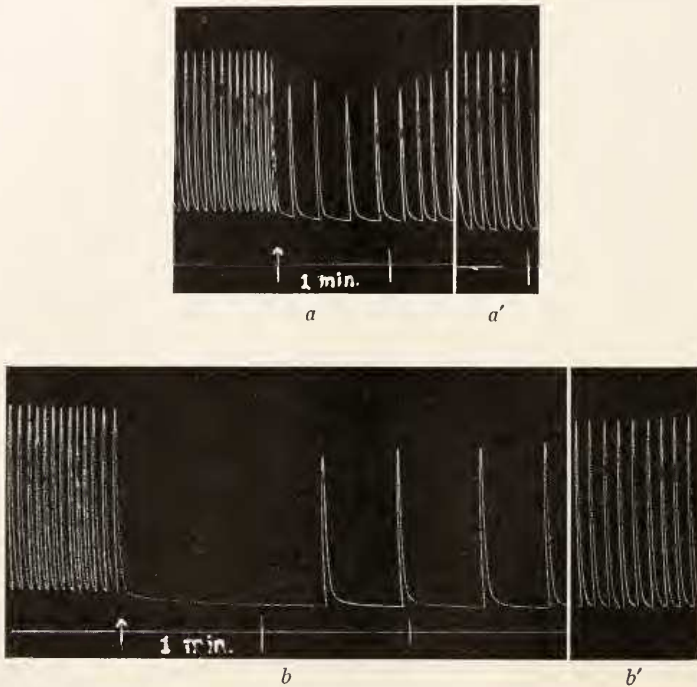


FIG. 1. Experiment No. 86. Effect of repeated immersion in the K-free Ringer's solution on the production of the K-paradox. (*a*) After a second immersion in a K-free Ringer's solution for ten minutes the ganglion, which did not give any sign of the K-paradox on the first trial, was returned to a normal Ringer's solution at the point marked by an arrow. (*a'*) Sixteen minutes later in the normal Ringer's solution. (*b*) K-paradox obtained on a similar immersion for the third time. (*b'*) Twelve minutes later in the normal Ringer's solution.

All figures read from left to right, and the change of solution is always marked by an arrow.

time. On transfer to the normal Ringer's solution the heart beat was immediately arrested for a minute and a half (Fig. 1, *b*).

In order to evaluate quantitatively the paradoxical effects of these two trials, the average rate for the first five minutes immediately following the change from the K-free to the normal Ringer's solution ( $R'$ ) was compared with the final rate in the latter ( $R$ ) when recovery is

complete. Expressed as the average percentage decrease in rate for the first five minutes following the change of solutions  $[(R - R')/R \times 100]$ , the results of these trials are given in Table I.

TABLE I

*Average percentage decrease in rate for the first five minutes on return to the normal Ringer's solution after repeated immersions in the K-free Ringer's solution. (Experiment No. 86.)*

Number of trials	Percentage decrease in rate
First.....	0
Second.....	31
Third.....	74

In this manner the intensity of the K-paradox is conveniently expressed in any experiment in terms of the primary decrease in rate. The greater the proportional decrease in rate, the more pronounced is the paradoxical effect.

Since the paradoxical effect is increased with the repeated immersion in the K-free Ringer's solution, a difficulty arises in comparing instances of K-paradox obtained under different conditions, even when the same ganglion is used. The difficulty may be obviated by performing the experiments in pairs, the order of the successive trials being reversed in the two or three trials of each pair.

TABLE II

*Average percentage decrease in rate for the first five minutes on return of the ganglion to normal Ringer's solution after immersions of unequal durations in K-free Ringer's solution.*

Duration of immersion in the K-free solution	Decrease in rate			Average
	Exper. No. 9	Exper. No. 10	Exper. No. 11	
<i>minutes</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
10	7.5	20	28	19
20	23	—	31	27
30	35	39	—	37

*The Effect of the Duration of Immersion in the K-free Ringer's Solution*

A certain minimum duration of immersion in the K-free Ringer's solution is necessary for the production of the K-paradox. This minimum duration, however, is difficult to determine with exactitude for the above-mentioned reason. On the other hand, if the exposure to the

K-free Ringer's solution is too long, so as to cause irreversible change in the ganglion, the rate and amplitude may continue to decrease on return to the normal Ringer's solution and no recovery will be seen. Within certain limits, up to an immersion of about thirty minutes, the changes are perfectly reversible in a good ganglion. Table II gives the comparison of the K-paradox obtained after immersions of 10, 20, and 30 minutes in the K-free Ringer's solution for three different ganglia.

In experiment No. 9 the order of experimentation was from shorter to longer durations; in experiments Nos. 10 and 11, from longer to shorter durations. As the duration of immersion in the K-free Ringer's solution increases, the decrease in rate becomes greater, and the primary decrease in amplitude is more pronounced; also the time for complete recovery is more prolonged.

#### *The Effect of the Difference in K-content in the Two Solutions*

Between the K-free and the normal Ringer's solution the difference of the K-content is about 9 millimols per liter, and the K-paradox usually appears on transfer to the normal Ringer's solution after an immersion of 10 minutes in the K-free solution. (In most of the experiments an immersion of ten minutes was adopted as the standard duration.) The paradox is also seen when a ganglion is transferred from the K-free solution to one containing an excess of KCl (*e.g.*, 18 millimols per liter). Although the excess of KCl by itself produces a rapid rhythm, yet the primary inhibition, particularly of the amplitude, is still obvious. In general, the paradoxical effect obtained on change from the K-free Ringer's solution increases, within certain limits, with the K-content of the second solution.<sup>4</sup>

When a K-poor Ringer's solution is used (*e.g.*, one containing 1 or 2 millimols KCl per liter) the K-paradox is still obtained on change to a normal Ringer's solution, or to one containing an excess of KCl. It is also obtained on change from a normal Ringer's solution to a Ringer's solution containing a large excess of KCl, about 70 to 100 millimols per liter. In general, a relatively great difference in the K-content of the two solutions is necessary for the production of the K-paradox, when the change is from a normal Ringer's solution to one containing an excess of KCl. The paradoxical effect is thus not directly proportional to the difference in the K-content. It is important to note that no such paradoxical effect is ever observed when the K-content is changed in the *decreasing* direction (*e.g.*, change from a K-excess to a normal Ringer's

<sup>4</sup> The K-paradox can also be observed on change to a Ringer's solution containing only 1 millimol KCl per liter after an immersion in the K-free Ringer's solution for twenty minutes.



solution, or from a normal to a K-poor Ringer's solution). The determining factor for the K-paradox is always the *sudden increase* in the K-content.

#### *The Effect of the Presence of Other Electrolytes*

When a fresh ganglion is immersed in a pure isotonic sucrose solution the rhythm can be maintained quite well for a considerable period up to half an hour; the precise duration depending upon the condition of the ganglion. If the ganglion thus active in pure sucrose solution is transferred to a sucrose solution containing KCl in the concentration normally present in the Ringer's solution, it is found that both the rate and the amplitude of the beat undergo a continuous decrease and the rhythm is gradually arrested in a few minutes; no K-paradox can be seen. This observation indicates that the presence of electrolytes other than KCl, either in the K-free or in the K-containing solution or in both, is necessary for the production of the K-paradox. There are thus two electrolytes (CaCl<sub>2</sub> and KCl) and two solutions (the K-free and the K-containing solutions) to be considered.

*Is CaCl<sub>2</sub> Necessary for the Production of the K-paradox?*—The K-paradox can be readily obtained on change from a pure isotonic NaCl solution to a normal Ringer's solution. Calcium is thus not necessary in the K-free solution. It is also not necessary in the K-containing solution, for the K-paradox is obtained on transfer from a K-free to a Ca-free Ringer's solution. The K-paradox appears on change from a pure isotonic NaCl solution to a Ca-free Ringer's solution (Fig. 2).

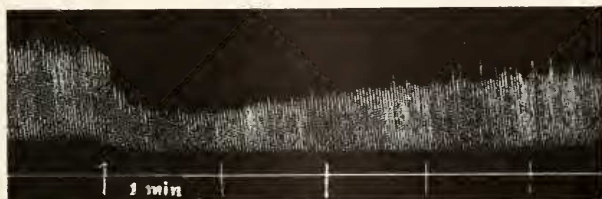


FIG. 2. Experiment No. 79. K-paradox obtained on change from a pure isotonic NaCl solution to the same containing 9 millimols KCl per liter (*i.e.*, the Ca-free Ringer's solution).

This last observation shows conclusively that CaCl<sub>2</sub> is not necessary for the production of the K-paradox and can be omitted from both the K-free and the K-containing solutions.

It should be noted, however, that in the last experiment cited, where the K-paradox appears on change from a pure NaCl solution to a Ca-

free Ringer's solution, the rate is only slightly inhibited for a short period and the primary decrease in amplitude may not appear at all. The difficulty with which the K-paradox is obtained under these circumstances is due to the fact that both the pure NaCl and the Ca-free solutions normally give rise to a rapid and irregular rhythm.

On the other hand, when an excess of  $\text{CaCl}_2$  (*e.g.*, 100 millimols per liter) is present in the K-free solution, the K-paradox can still be obtained on return to the normal Ringer's solution if the rate and amplitude are not unduly decreased while the ganglion is in the former solution. Similarly, the K-paradox still can be obtained on change from a K-free Ringer's solution to a normal Ringer's solution containing an excess of  $\text{CaCl}_2$  (100 millimols per liter), provided the  $\text{CaCl}_2$  does not reach such a concentration as to inhibit the rhythm by itself (Fig. 3). Finally the K-paradox has been obtained on change from a K-free to a



FIG. 3. Experiment No. 92. K-paradox obtained on change from a K-free Ringer's solution to a normal Ringer's solution containing an excess of  $\text{CaCl}_2$  (100 millimols per liter).

K-containing Ringer's solution, both containing the same excess of  $\text{CaCl}_2$  (80 millimols per liter). It seems, therefore, that the K-paradox is not abolished by the removal of  $\text{CaCl}_2$ , nor is it antagonized by the excess of  $\text{CaCl}_2$ , and that the general effect of  $\text{CaCl}_2$  is only secondary, consisting in balancing part of the toxic action of NaCl.

*Is NaCl Necessary for the Production of the K-paradox?*—Since the presence of some other electrolyte either in the K-free or in the K-containing solution has been shown to be necessary for the production of the K-paradox, and since  $\text{CaCl}_2$  has been shown to be unnecessary, the logical conclusion follows that NaCl must be the necessary electrolyte. In fact, the paradoxical effect is found to be progressively diminished with progressive decrease in the NaCl-content of the K-free solution. When a fresh ganglion is immersed in a mixture of 30 volumes of a K-free Ringer's solution<sup>5</sup> and 70 volumes of isotonic sucrose

<sup>5</sup> K-free Ringer's solution is used instead of pure NaCl solution on account of the less toxic nature of the former.

solution, hardly any sign of the K-paradox can be demonstrated on return to the normal Ringer's solution. The K-paradox is obtained only on a second or third trial with such a solution, and the paradoxical effect is always less marked than that obtained with a 100 per cent K-free Ringer's solution. Table III gives the results of the primary decrease in rate for four experiments. In these experiments the first trials with either the 100 per cent or the 30 per cent K-free Ringer's solution were discarded, and in two experiments the order of treatment with the two solutions was reversed for the purpose of control, as already described.

When NaCl is entirely replaced by sucrose in the K-free solution the K-paradox is entirely abolished. No paradoxical effect has ever been

TABLE III

*Average percentage decrease in rate for the first five minutes on change to normal Ringer's solution after an immersion of ten minutes in (1) a 100 per cent and (2) a 30 per cent K-free Ringer's solution.*

Experiment No.	Decrease in 100 per cent solution per cent	Decrease in 30 per cent solution per cent
75	36	29
76	36	20
77	33	18
78	54	16
Average	40	21

observed on change from a pure isotonic sucrose solution, or from the same solution containing 37 millimols  $\text{CaCl}_2$  in a liter, to the normal Ringer's solution.

NaCl, however, is necessary only in the K-free Ringer's solution, for the K-paradox can be obtained on change from a pure isotonic NaCl

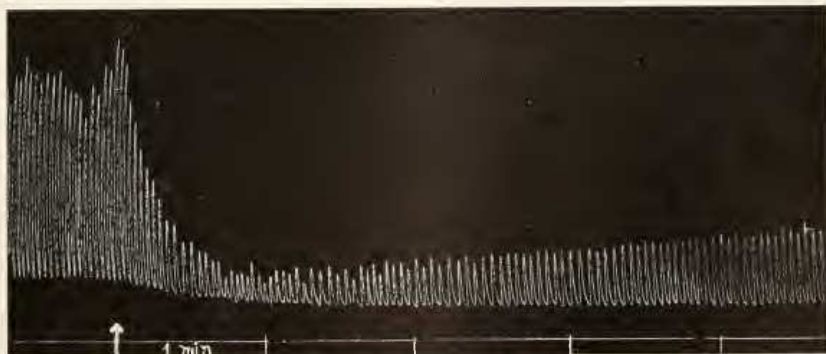


FIG. 4. Experiment No. 63. K-paradox obtained on change from a pure isotonic NaCl solution to a sucrose solution containing 9 millimols KCl per liter.



solution to a sucrose solution containing KCl in the concentration normally present in Ringer's solution <sup>6</sup> (Fig. 4).

### *The Calcium-paradox*

When a ganglion is immersed in a Ca-free Ringer's solution for several minutes the rate of the heart-beat is accelerated considerably. If then the Ca-free Ringer's solution is replaced by a normal Ringer's solution, the rate falls progressively to normal (rapidly at first and more gradually later), while the amplitude undergoes a somewhat rapid decrease to a minimum height followed by a period of gradual rise to normal (Fig. 5). This primary and temporary decrease in amplitude on change from a Ca-free to a normal Ringer's solution resembles that observed in the K-paradox, though it is less pronounced, and is known

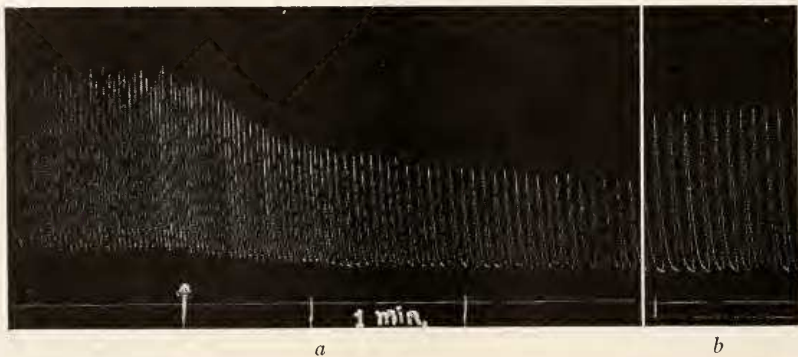


FIG. 5. Experiment No. 26. (a) Ca-paradox obtained on change from a Ca-free to a normal Ringer's solution after the ganglion has been immersed in the former for twenty minutes. (b) Eighteen minutes later in the normal Ringer's solution.

as the Ca-paradox. Like the K-paradox, it is more readily obtained on a second or a third repetition than on the first immersion of a fresh ganglion in the Ca-free Ringer's solution; and the primary decrease in amplitude is greater after a longer period of immersion in the Ca-free Ringer's solution. The change illustrated in Fig. 5, for instance, was obtained after an immersion of a fresh ganglion in the Ca-free Ringer's solution for twenty minutes; a second immersion for ten minutes gave a very small primary decrease in amplitude indeed.

The Ca-paradox differs from the K-paradox essentially in two respects. In the first place, the primary temporary inhibition affects the amplitude only; during the process of recovery, the rate returns pro-

<sup>6</sup> The K-paradox can be more readily demonstrated on change from a K-free Ringer's solution to a sucrose solution containing KCl.

gressively to normal without first decreasing to a minimum. Since the amplitude is not always a good criterion for quantitative evaluation of the changes (*e.g.*, the amplitude is often markedly irregular in such a solution), and since the Ca-free Ringer's solution is more toxic than the K-free Ringer's solution, the Ca-paradox is not so easily obtained as the K-paradox. For this reason the observations on the Ca-paradox have been relatively few. In the second place, it seems that the Ca-paradox is obtained only on change from a Ca-free to a normal Ringer's solution. It is not obtained on change from a Ca-free Ringer's solution to a sucrose solution containing 37 millimols  $\text{CaCl}_2$  in a liter, or to a K-free Ringer's solution.<sup>7</sup> Furthermore, the presence of an excess of  $\text{CaCl}_2$  in the second solution prevents the appearance of any change of the paradoxical kind; there is simply a progressive decrease in rate and amplitude. In short, the Ca-paradox is not proportional to the difference in the Ca-content of the two solutions and requires the presence of both NaCl and KCl.

#### *The Thermo-paradox*

The rate of the heart-beat of *Limulus* is always increased at a higher temperature and decreased at a lower temperature. Therefore, on sud-

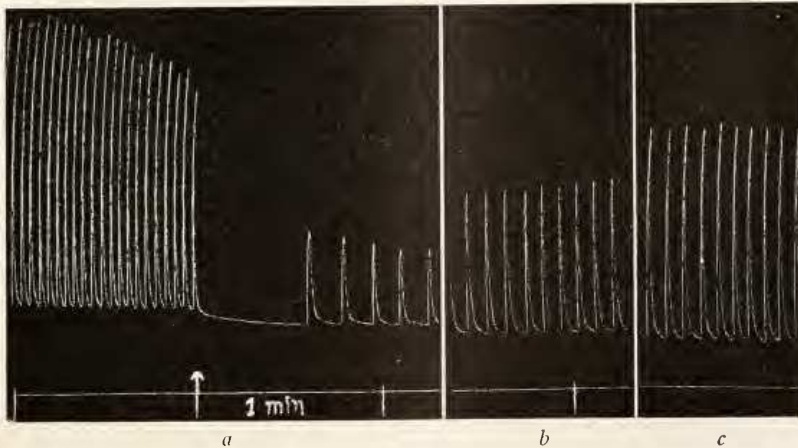


FIG. 6. Experiment No. 32. (a) Thermo-paradox obtained on change from a warm Ringer's solution at  $41.5^\circ \text{C}$ . to another Ringer's solution at room temperature ( $24.0^\circ \text{C}$ ). (b) Seven minutes later; and (c) 14 minutes later at the room temperature.

denly bringing a ganglion from a Ringer's solution at the room temperature to a warm Ringer's solution, the rate is almost instantaneously in-

<sup>7</sup> The changes obtained on transfer from a pure NaCl solution to a normal Ringer's solution resemble the K-paradox rather than the Ca-paradox, for usually both the rate and the amplitude undergo the primary and temporary decrease.

creased. On bringing it back to the room temperature, the rate would be expected to return either rapidly, or gradually, to normal. What actually happens, however, is of the general nature designated as paradoxical—both the rate and the amplitude are immediately decreased to a level below normal, or the heart-beat may be completely arrested for a short period; then the normal rhythm for the room temperature is gradually resumed. This primary and temporary inhibition of the cardiac rhythm on change from a high to a low temperature is called the thermo-paradox. In general this effect is more pronounced the greater the decrease in temperature. When the temperature was suddenly decreased from  $41.5^{\circ}$  to  $24.0^{\circ}$  (Fig. 6), the heart-beat was immediately arrested for about half a minute and a gradual recovery followed. When the temperature was then decreased from  $33.5^{\circ}$  to  $24.0^{\circ}$  on the same ganglion, the heart-beat was greatly decreased both in rate and in amplitude, but not completely arrested, and the recovery was complete in a relatively short period.

#### DISCUSSION

The demonstration of K-paradox, Ca-paradox and thermo-paradox in the ganglion of the *Limulus* heart as well as in the vertebrate heart offers additional evidence of the general physiological similarity between these hearts. The paradoxes observed in the ganglion of the *Limulus* heart differ, however, from those of the vertebrate heart in that while in the former both rate and amplitude may be involved in the primary temporary inhibition, in the latter the primary inhibition affects the rate only. This difference is to be expected when one considers that the vertebrate heart is strictly of the all-or-none type; while in the *Limulus* heart the amplitude of the contractions may vary greatly, apparently in correlation with the number of nerve cells acting simultaneously in the ganglion. A decrease in amplitude means simply a decrease in the number of active nerve cells. With the exception of this apparent difference and a few minor points, the paradoxes found in the ganglion of the *Limulus* heart are essentially identical with those described for the vertebrate heart.

Many explanations have been suggested for the paradox phenomena. In connection with his work on physiological radioactivity Zwaardemaker (1917, 1921) attempted to explain his radio-physiologic paradoxes in terms of the antagonistic actions between the light and the heavy radioactive substances when applied successively. Libbrecht believed that the K-paradox is a specific K-effect connected with the ability of the tissue to adapt itself to changes in environmental conditions. In his paper on the rabbit's heart, Busquet pointed out certain resem-

blances between the K-paradox and the effects of vagus stimulation. Yet the K-paradox can still be produced after the action of the vagus nerve is abolished by atropine (Busquet, 1921, and Kisch, 1927). Witanowski (1926) regarded a change in the permeability of the cell membrane to K-ion in the K-free solution as the essential factor in the production of the K-paradox. But the Ca-paradox, Sr-paradox, and thermo-paradox cannot be satisfactorily explained on these theories; and the demonstration of these paradoxes in the ganglion of the *Limulus* heart and in other tissues with automatic rhythmic activity indicates that the phenomenon is a general one, and that some general, or unitary mechanism is involved. Two distinct and equally important factors appear to be concerned: (1) a preliminary process in a modified medium, and (2) a process connected with the sudden return to the normal medium. The paradox appears almost simultaneously with the change of the solutions, indicating apparently a surface phenomenon. The preliminary process (*e.g.*, the immersion in a K-free, or a Ca-free, or a warm Ringer's solution) is preparatory in nature and requires a minimum duration; its effect is to establish a new equilibrium in the tissue such that on subsequent return to its normal medium a primary and a purely temporary inhibition of activity occurs before the tissue can resume its normal condition, *i.e.*, during the temporary period of disequilibrium in the second solution. This is the essence of the paradox phenomenon.

#### SUMMARY

Potassium-paradox, calcium-paradox, and thermo-paradox have been observed in the ganglion of the *Limulus* heart.

The Ca-paradox is obtained on change from a Ca-free to a normal Ringer's solution, and the thermo-paradox is obtained on change from a warm to a cold Ringer's solution.

The K-paradox is obtained in a similar way on change from a K-free to a normal Ringer's solution, and its production depends upon the following factors:

1. The K-paradox is more readily obtained on the second or third repetition than on the first immersion of a fresh ganglion in the K-free Ringer's solution.
2. The paradoxical effect becomes more pronounced with increasing duration of the preliminary immersion in the K-free Ringer's solution.
3. The factor determining the K-paradox is the sudden change in the K-content of the medium surrounding the ganglion, and this change must be in the direction of increasing the K-content in the second solution.

4. The presence of a minimum amount of NaCl in the K-free Ringer's solution (about 30 per cent of the normal concentration) is necessary for the production of the K-paradox. The presence of CaCl<sub>2</sub> in the K-free, or the K-containing, solution is of only secondary importance perhaps by virtue of its antagonistic action.

In general the paradoxes found in the ganglion of the *Limulus* heart are essentially identical with those described in the vertebrate heart.

The author wishes to express his profound gratitude to Dr. R. S. Lillie for invaluable advice and much help.

## REFERENCES

- BAKKER, B., 1927. Onderzoekingen in het physiol. laborat. d. Rijksuniversiteit te Utrecht, **7**: 129.
- BUSQUET, H., 1921. *Compt. rend. Soc. de Biol.*, **85**: 1142.
- BUSQUET, H., 1922a. *Compt. rend. Soc. de Biol.*, **86**: 106.
- BUSQUET, H., 1922b. *Compt. rend. Soc. de Biol.*, **86**: 1010.
- CHAO, I., 1933. *Biol. Bull.*, **64**: 358.
- HOBSON, A. D., 1928. *Brit. Jour. Exper. Biol.*, **5**: 385.
- KISCH, B., 1927. *Arch. f. exper. Pathol. u. Pharmacol.*, **122**: 257.
- KISCH, B., 1930. *Arch. f. exper. Pathol. u. Pharmacol.*, **148**: 140.
- LIBBRECHT, W., 1920. *Arch. internat. de physiol.*, **15**: 446.
- LIBBRECHT, W., 1921. *Arch. internat. de physiol.*, **16**: 448.
- WELLS, G. P., 1928. *Brit. Jour. Exper. Biol.*, **5**: 258.
- WITANOWSKI, W. R., 1926. *Pflüger's Arch.*, **212**: 726.
- ZWAARDEMAKER, H., 1917. *K. Akad. v. Wetensch. Amsterdam*, **19**: 1043.
- ZWAARDEMAKER, H., 1921. *Ergebn. d. Physiol.*, **19**: 326.
- ZWAARDEMAKER, H., 1925. *Ergebn. d. Physiol.*, **24**: 166.